REMARKS

An Office Action was mailed in the above-captioned application on November 10, 2003. In such Office Action claims 1-10 were pending. Claim 9 was rejected under 35 U.S.C. § 112 (first paragraph). Claims 1-8 and 10 were rejected under 35 U.S.C. § 103(a). This Amendment and Remarks is submitted in response to such Office Action.

Claim Rejection – 35 U.S.C. § 112, ¶1

Claim 9 was rejected under 35 U.S.C. § 112, first paragraph. In the interests of furthering prosecution of this application, claim 9 has been cancelled without prejudice as to the subject matter contained therein. Applicant reserves the right to pursue the cancelled subject matter in a continuing (or subsequent related) application.

Claim Rejections – 35 U.S.C. § 103(a)

A. Claims 1-4

Claims 1-4 were rejected under 35 U.S.C. § 103(a) as obvious over Newby et al., *Rapid Commun. Mass Spectrom* 11:1723-1727 (1997) ("Newby et al.") in view of Schilling et al., *Biomed. Environ. Mass Spectrom.* 10:545-51 (1986) ("Schilling et al.").

Claims 1-4 recite a method for performing liquid chromatography-mass spectrometry on a chemical mixture comprising a least two prostaglandins. The claims require (i) adding a basic liquid to the HPLC eluent (ii) using sheath flow to (iii) generate a diluted eluent and (iv) performing mass spectrometry on the diluted eluent. The enhanced separation and increased ionization efficiency provided by the method allows the prostaglandin species to be separated and identified at very low concentrations. See, e.g., Specification at page 3, lines 10-19.

Newby et al. describes a method for quantitative analysis of a number of lipid mediators using a high performance liquid chromatograph (HPLC) directly coupled to an electrospray ionization (ESI) mass spectrometer. (Newby et al. at page 1723, col. 1; page 1724, col. 2 - page 1725, col. 1). The components of the mixture are separated on the HPLC column using an acidic mobile phase (acetonitrile with aqueous formic acid) that is directly injected into the mass

spectrometer. Thus, both the chromatographic separation and the electrospray ionization are performed using the same acidic media (page 1724, col. 1 - page 1725, col. 1).

Newby et al. does not teach or suggest adding a basic liquid to the HPLC eluent. Newby et al. does not teach or suggest using sheath flow to add a liquid of any kind to the eluent. Newby et al. does not teach or suggest diluting the eluent. Newby et al. does not teach or suggest performing mass spectrometry on a diluted eluent.

Schilling et al. does not remedy the deficiencies of Newby et al. Shilling et al. describes a method for analyzing prostglandins using direct chemical ionization (DCI) mass spectrometry. [1] According to the method, the sample containing the analyte is dried on a probe tip and the probe is inserted into the mass spectrometer in an atmosphere of ionized reagent gas (plasma). Using an electric current, the probe is rapidly heated to desorb the analyte molecules. Once in the gas phase, the analyte molecules are ionized through collisions with ionized reagent gas molecules. See Shilling et al. at page 547, col. 1.

The Examiner contends that, in view of Schilling et al., it would have been obvious for one of skill in the art to modify the HPLC/ESI mass spectrometry method of Newby et al. by adding an ammonia hydroxide solution to the HPLC eluent before it is analyzed by mass spectrometry. Schilling et al. never mentions ammonium hydroxide solution, or adding a basic liquid of any kind to an eluent. However, the Examiner asserts that "Schilling et al. demonstrated that performing MS analysis in the atmosphere of NH_3 allows detecting prostaglandin isomers, including PGE_2 and PGD_2 without prior derivatization" and that "[a]dding basic NH_4OH solution to the HPLC eluent prior to MS analysis is similar to adding NH_3 gas to the mixture in Shilling et al.'s method." (Office Action at page 4, first full paragraph).

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[1] The Examiner states that Schilling et al. were able to distinguish between arachidonic acid metabolites using NH_3 as the reagent gas. However, in the passage quoted by the Examiner, the "latter reagent gas" refers to tri-deuterated ammonia ($N\ ^2H_3$), not ammonia (NH_3). Schilling et al. at page 545 (abstract). Schilling et al. was able to distinguish PGH_2 from PGD_2 using tri-deuterated ammonia because the former has only two exchangeable protons while the latter has three. Schilling et al. at page 550, col. 1. However, Shilling et al. does not purport to be able to distinguish PGD_2 and PGE_2 from each other using this technique because they each have the same number of exchangeable protons.

For the reasons set forth below, neither of these assertions is sound.

A mass spectrometer works by using magnetic and electric fields to exert forces on charged particles (ions) in a vacuum. Therefore, a compound must be charged or ionized to be analyzed by a mass spectrometer. Furthermore, the ions must be introduced in the gas phase into the vacuum system of the mass spectrometer.

DCI uses ion-molecule reactions to generate ions from the sample. The process begins when a reagent gas, such as ammonia, is ionized by electron impact. In Schilling et al., for instance, the ammonia gas is ionized by electron beam bombardment at an energy of 70 eV (Shilling et al. at page 547, col. 1). The sample containing the analyte is placed on a filament that is rapidly heated to desorb the analyte molecules into the ionized reagent gas. The desorbed analyte molecules react in the gas phase by colliding with the ionized gas to produce analyte molecule ions. Accordingly, Shilling reports detecting $[M + NH_4]^+$ and $[M + N_2H_7]^+$ adducts formed between analyte molecules and ionized gas molecules. See Shilling et al., Figure 3 (positive ion, ammonia DCI mass spectrum of PGE_2, for example, has a peak at 370 corresponding to the NH_4^+ adduct and a peak at 387 corresponding to the $N_2H_7^+$ adduct).

By contrast, in a basic liquid (e.g., an NH_4OH solution), which is what is claimed in the present application, prostaglandins exist in solution as deprotonated prostaglandin ions. Such $[M - H]^-$ ions provide increased ionization efficiency in the negative mode. According to the teachings of the present invention, "the chromatography is performed under acidic conditions to enhance separation, while the spectrometry is performed under basic conditions to increase ionization efficiency in the negative ion mode." Specification at page 4, lines 31-32. This is accomplished in the present invention by introducing a basic solution between the two stages. Optimizing both chromatographic separation and ionization efficiency allows prostaglandins to be separated and identified at very low concentrations.

Significantly, neither Schilling et al. nor Newby et al. recognizes even the possibility of the advantages provided by the present invention. The references, either alone or in combination, do not teach or suggest that it would be desirable to add a basic liquid to the HPLC eluent in the technique of Newby et al. so that, in an illustrative embodiment, an acidic eluent is used for chromatographic separation while a (diluted) basic eluent undergoes electrospray ionization.

Shilling et al. does not disclose adding a liquid, basic or otherwise, to an eluent. Newby et al. report that "[a]n intense $[M - H]^-$ anion was readily detected for all the chosen lipid mediators" using the acidic mobile phase (Newby et al. at page 1725, col. 2). In fact, Newby et al. states "we observed no reduction in the signal obtained for the lipid mediators with our without the presence of the formic acid" (Newby et al. at page 1727, col. 1). Thus, one of skill in the art, having both of the references before him or her, would not have been motivation to modify the technique of Newby et al., or to combine their teachings in the manner suggested by the Examiner.

Furthermore, neither reference teaches or suggests that the results obtained in gas phase with ammonia reagent gas using DCI can be translated to solution phase reactions with ammonium hydroxide solution using ESI. Absent such a teaching, especially in an unpredictable art, one of skill in the art would have no motivation to combine the references or reasonable expectation that the combination would be successful.

Indeed, both references describe their respective techniques as being applicable to further studies of the same type. Shilling et al. states that "DCI mass spectrometry using NH_3 ($N\ ^2H_3$) reagent gases should prove useful in the analysis of AA metabolites without the need for prior derivatization." Schilling et al. at page 550, col. 2. Similarly, Newby et al. states that "HPLC/ESI-MS proved to be a powerful tool for the quantative analysis of lipid inflammatory mediators in cultured fibroblasts" and that "[t]he procedure can be applied to other important lipid mediators ... and may be applicable to other cell culture experiments" (Newby et al. at page 1727, col. 1).

Moreover, even if one of skill in the art were so motiviated, neither reference teaches or suggests how the references could be combined. Neither reference discloses sheath flow, or any means for that matter, by which a basic liquid could be introduced in the HPLC eluent before mass spectrometry analysis.

Finally, even if the references were combined in the manner suggested by the Examiner, they would not teach or suggest all the claim limitations. Neither Newby et al. nor Schilling et al. teach or suggest diluting an HPLC eluent, performing mass spectrometry on a diluted eluent,

adding a basic liquid to an HPLC eluent, or using sheath flow to add a liquid of any kind to the eluent.

Applicant submits that one of skill in the art would not have found the claimed invention to be obvious in light of the teachings of the references. The rejection should be withdrawn.

B. *Claim 5*

Claim 5 was rejected under 35 U.S.C. § 103(a) over Newby et al. in view of Schilling et al. and further in view of Margalit et al., *Anal. Biochem.* 1:73-81 (1996) ("Margalit et al.").

The Examiner acknowledges that neither Newby et al. nor Schilling et al. disclosure tandem mass spectrometry for the analysis of prostaglandins. However, the Examiner states that Margalit et al. discloses the use of detecting the advantages of using ESI-MS/MS in the detection of prostaglandin metabolites. The Examiner concludes that it would have been obvious for anyone of ordinary skill in the art to use tandem mass spectrometry in the "Newby-Schilling" method because Margalit et al. demonstrates the advantages of ESI-MS/MS in the detection of prostaglandin metabolites.

As described above, the invention of claims 1-4 are not obvious over Newby et al. in view of Schilling et al. Margarlit et al. does not remedy those deficiencies. Thus, the invention of claims 5 is not obvious over Newby et al. in view of Schilling et al. and Margalit et al. for at least the same reasons that the invention of claims 1-4 are not obvious over Newby et al. in view of Schilling et al.

C. *Claim 6*

Claim 6 was rejected under 35 U.S.C. § 103(a) over Newby et al. in view of Schilling et al., Margalit et al., and further in view of either Ballard et al., *Rapid Comm. Mass Spectrom.* 6:553-559 (1992) ("Ballard et al.") or Kanai et al., (Japanese) *Kuromatogurafi* 17:162-163 (1996) ("Kanai et al.").

The Examiner acknowledges that Newby et al. in view of Schilling et al. and Margalit et al. does not teach MS^4 tandem mass spectrometry. However, the Examiner states that both Ballard et al. and Kanai et al. disclose instrumental improvements of MS spectrometers, which give the

opportunity to perform more informative MS^3 and MS^4 experiments, which Kanai et al. states are "very useful for the structural analysis of biomolecules."

The Examiner concludes that it would have been obvious for anyone of ordinary skill in the art to apply improved and more sophisticated MS^4 experiments for analysis of prostaglandins, because MS^4 spectra are highly informative for biomolecules, as indicated by Kanai et al.

As described above, the invention of claims 1-5 are not obvious over Newby et al. in view of Schilling et al. and Margarlit et al. Neither Ballard et al. nor Kanai et al. remedies those deficiencies. Thus, the invention of claim 6 is not obvious over Newby et al. in view of Schilling et al., Margalit et al. and further in view of Ballard et al. or Kanai et al. for at least the same reasons that the invention of claims 1-5 are not obvious over Newby et al. in view of Schilling et al. and Margarlit et al.

D. *Claims 7-8 and 10*

Claims 7-8 were rejected under 35 U.S.C. § 103(a) over Margalit et al. in view of Bomse et al., U.S. Patent No. 5,015,848 ("Bomse et al."). Claim 10 was rejected under 35 U.S.C. § 103(a) over Margalit et al. in view of Bomse et al. and further in view of Ballard et al. or Kanai et al.

In the interests of furthering prosecution of this application, claims 7-8 and 10 have been cancelled without prejudice as to the subject matter contained therein. Applicant reserves the right to pursue the cancelled subject matter in a continuing (or subsequent related) application.

New Claims

New claim 11 depends from claim 1 and further specifies that the prostaglandins are isobaric. Support for new claim 11 is found, e.g., at page 4, lines 18 and 21. New claim 12 depends from claim 1 and further specifies that the prostaglandins are isomers. Support for new claim 12 is found, e.g., at page 4, line 18. New claim 13 depends from claim 1 and further specifies that performing the mass spectrometry comprises performing mass spectrometry in the negative mode. Support for new claim 13 is found, e.g., at page 4, line 32. New claim 14 depends from claim 1 and further specifies that the basic solution comprises ammonium hydroxide. Support for new claim 14 is found, e.g., at page 5, lines 8 and 26. New claim 15 depends from claim 1

and further specifies that the basic solution comprises acetonitrile. Support for new claim 15 is found, e.g., at page 5, lines 8, 17 and 27. New claim 16 depends from claim 1 and further specifies that the eluent comprises acetic acid. Support for new claim 16 is found, e.g., at page 5, line 17. New claim 17 depends from claim 1 and further specifies that the eluent comprises acetonitrile. Support for new claim 17 is found, e.g., at page 5, line 17.

New claims 11-17 are patentable over the prior art for the same reasons as claim 1 from which they depend.

New claim 18 is an independent claim that recites the same method as claim 1, except that it does not contain the sheath flow limitation. Support for new claim 18 is found, e.g., at page 3, lines 10-19; page 4, lines 26-32; and originally filed claim 1.

New claims 19-30 are the same as originally field claims 2-6 and new claims 11-17. New claims 19-23 share support with originally filed claims 2-6 and new claims 24-30 share support with new claims 11-17, as set forth above.

New claims 19-30 are patentable over the prior art for the same reasons as originally filed claims 2-6 and new claims 11-17, as set forth above.

Closing Remarks

Applicant believes that the pending claims are in condition for allowance. If it would be helpful to obtain favorable consideration of this case, the Examiner is encouraged to call and discuss this case with the undersigned.

This constitutes a request for any needed extension of time and an authorization to charge all fees therefore to deposit account No. 19-5117, if not otherwise specifically requested. The undersigned hereby authorizes the charge of any fees created by the filing of this document or any deficiency of fees submitted herewith to be charged to deposit account No. 19-5117.

Respectfully submitted,

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